LEVEL II

12
6





DEPARTMENT OF STATISTICS

# The Ohio State University

OSU

COLUMBUS, OHIO

12

# ESTIMATION OF RATE CONSTANTS IN THE MICHAELIS-MENTEN MODEL

J. S. Rustagi* and Joanne Yang

Department of Statistics
The Ohio State University
Columbus, Ohio 43210



Technical Report No. 177

March 1979



*Supported in part by Contract N00014-78-C-0543 (NR 042-403) by Office of Naval Research. Reproduction in whole or in part is permitted for any purpose of the United States Government.

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(14) TR-177

## REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 177
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): (6) Estimation of rate constants in the Michaelis - Menten Model.

5. TYPE OF REPORT & PERIOD COVERED: (9) Technical Report

6. PERFORMING ORG. REPORT NUMBER: 177

7. AUTHOR(s): (10) J. S. Rustagi & Joanne Yang

8. CONTRACT OR GRANT NUMBER(s): (15) N00014-78-C-0543

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Department of Statistics
Ohio State University
1958 Neil Ave. Columbus, Ohio 43210

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 042-403

11. CONTROLLING OFFICE NAME AND ADDRESS
Office of Naval Research
Department of Navy
Arlington, Virginia 22207

12. REPORT DATE: (11) March 1979

13. NUMBER OF PAGES: 15

14. MONITORING AGENCY NAME & ADDRESS (If different from Controlling Office): (12) 18p.

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
This document has been approved for public release and sale; its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

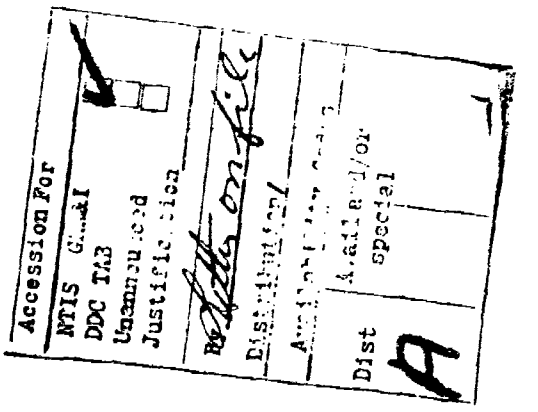
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Rate Constants, Michaelis - Menten equation, Compartment Models, Nonlinear estimation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Nonlinear models leading to Michaelis - Menten scheme result from the study compartmental systems as well as in other contexts. Using difference equation approach, rate constants are estimated in some simple models. Simulation methods are used to derive the distributions of these estimates. It is shown that the assumptions of normality of errors leads to the normal distribution of the estimated rate constants.

406 331

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102 LF-014-6601

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)




# 1. INTRODUCTION

Non-linear models arise in many practical applications. Models based on Michaelis-Menten differential equations are commonly used in biochemistry, biopharmaceutics, pharmacokinetics and other related disciplines. These differential equations result in general when compartmental models are applied. Linear differential equations usually suffice for the study of compartmental models with constant rates of exchange among compartments. However, there are many situations especially in the study of drug distribution where nonlinearity arises naturally. Several methods have been proposed in the literature to fit constants in such nonlinear models. In an earlier paper, Rustagi and Singh (1977) used difference equation approach to fit one- and two-compartment models under the assumptions of linear kinetics.

In this paper, the Michaelis-Menten elimination scheme is studied using the difference equation approach. The distribution of the estimates of the resulting rate constants is not easily obtainable in closed form. Using Monte-Carlo methods, the distribution of these constants is derived for the special case of normal errors. Similar methods can be utilized under different distributional assumptions for errors in the model.

Applications of Michaelis-Menten equations to pharmacokinetics has recently been made by Wagner (1973) and Sedman and Wagner (1974).

They obtained estimates of the rate constants by numerical methods but did not provide the distribution of these estimates. It is well known that it is not easy to obtain the distribution of estimates which are obtained through numerical techniques. These distributions are, however, necessary for statistical analysis, for example, in obtaining the confidence interval estimates for the rate constants or in comparing two different rate constants.

## 2. MICHAELIS-MENTEN MODELS

In a study dealing with enzyme kinetics, Michaelis and Menten (1913) provided a model for the following one compartment system, given by Figure 2.1.

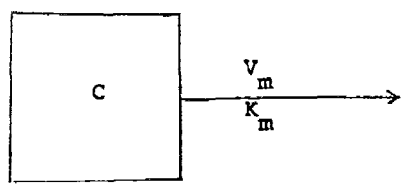



Figure 2.1

C is the concentration of certain substance in the compartment, $K_m$ is the rate of output and $V_m$ is the maximum rate of reaction. This system can be represented by the differential equation

$$\frac{dC}{dt} = -\frac{V_m C}{K_m + C} . \qquad (2.1)$$

Consider the difference equation analog of the equation (2.1) assuming that the process is observed at times $t_1, t_2, \ldots, t_n$. Using the

approximation for derivatives in the case of unequal time intervals, we have

$$\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = -\frac{V_m C(t_i)}{K_m + C(t_i)} . \tag{2.2}$$

Equation (2.2) is simplified in the following form.

$$K_m \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = 0 . \tag{2.3}$$

The model for estimating constants $K_m$ and $V_m$ is assumed to be

$$K_m \frac{C(t_{i+1})-C(t_i)}{t_{u+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = u(t_i) . \tag{2.4}$$

We assume for simplicity that $u(t_i)$ are random errors with zero means and variances $\sigma_i^2$.

The weighted least-squares solution for $K_m$ and $V_m$ are obtained by minimizing the expression,

$$S(V_m,K_m) = \sum_{i=1}^{n-1} \frac{1}{\sigma_i}\left[K_m \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i}\right]^2 , \tag{2.5}$$

where $\frac{1}{\sigma_i}$ is the weight for each data point. By taking the derivatives of equation (2.5) with respect to $K_m$ and $V_m$, we get the normal equations given in (2.6) and (2.7) which provide the least-squares estimates for $K_m$ and $V_m$.

$$\hat{K}_m \sum_{i=1}^{n-1} \frac{1}{\sigma_i} \left( \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} \right)^2 + \hat{V}_m \sum_{i=1}^{n-1} \frac{1}{\sigma_i} C(t_i) \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i}$$

$$+ \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \left( \frac{C(t_{i+1})-Ct_i)}{t_{i+1}-t_i} \right)^2 = 0 \qquad (2.6)$$

$$\hat{K}_m \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \cdot \frac{C(t_{i+1})-Ct_i)}{t_{i+1}-t_i} + \hat{V}_m \sum_{i=1}^{n-1} \frac{(C(t_i))^2}{\sigma_i}$$

$$+ \sum_{i=1}^{n-1} \frac{(C(t_i))^2}{\sigma_i} \cdot \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = 0 \qquad (2.7)$$

Example

Suppose the time-concentration data of alcohol elimination in human subject is given in Table 2.1. The data is fitted to (2.4) by using $\frac{1}{(C(t_i))^2}$ as the weights, we get $K_m$ = 2.8021 mM, and $V_m$ = 0.0882 mM/min. The ratio $V_m/K_m$ is 0.0315 $min^{-1}$. This ratio is close to the theoretical approximate first-order rate constant for drug elimination which is known to be 0.0513 $min^{-1}$, Wagner (1971).

Table 2.1

| Time (min) | 5.0 | 48.0 | 78.0 | 105.0 | 135.0 | 163.0 |
|---|---|---|---|---|---|---|
| Concen. (mM) | 6.9 | 4.1 | 2.7 | 1.4 | 0.5 | 0.15 |

Note that the distribution of the estimates $\hat{K}_m$ and $\hat{V}_m$, which depend non-linearly on $C(t_i)$, are not easily obtainable for given n. Simulation methods are used later to obtain the properties of these estimates.

The characteristics of the two-compartment model with Michaelis-Menten kinetics have been studied by several authors, for example see Sedman and Wagner (1974). Suppose a drug is injected into a biological system intravenously. This system can be represented by a two-compartment open model where elimination occurs from the first compartment. A schematic diagram is given in Figure 2.2 using nonlinear kinetics.

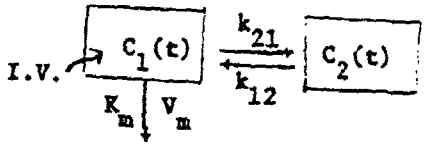



Figure 2.2

Here $C_1(t)$ and $C_2(t)$ are the concentrations in the two compartments, $k_{12}$ and $k_{21}$ are the first-order rate constants, and $K_m$, $V_m$ have the same meaning as before. The mathematical model for concentrations results in the equations (2.8) and (2.9).

$$\frac{dC_1}{dt} = -(k_{21} + \frac{V_m}{K_m + C_1})C_1 + k_{12}C_2 \ , \tag{2.8}$$

$$\frac{dC_2}{dt} = k_{21}C_1 - k_{12}C_2 \ . \tag{2.9}$$

Suppose an experimenter can observe the concentration in only one compartment. From (2.8) we have

$$k_{12}C_2 = \frac{dC_1}{dt} + k_{21}C_1 + \frac{V_m}{K_m + C_1}C_1 \ . \tag{2.10}$$

Substituting (2.10) into (2.9), we get

$$\frac{dC_2}{dt} = -\frac{dC_1}{dt} - \frac{V_m}{K_m + C_1} . \tag{2.11}$$

Differentiating equation (2.8) once more, we have a second-order differential equation in terms of $C_1$,

$$\frac{d^2C_1}{dt^2}(K_m+C_1)^2 + [(K_m+C_1)^2(k_{21}-k_{12}) - V_mK_m]\frac{dC_1}{dt} + k_{12}V_m(K_m+C_1)C_1 = 0 . \tag{2.12}$$

Suppose now that the concentration in a given compartment is observed at equal intervals of time. For notational convenience we drop the subscript in $C_1$ and replace the derivative $\frac{dC}{dt}$ by $C(t+1) - C(t)$ and $\frac{d^2C}{dt^2}$ by $C(t+2) - 2(t+1) + C(t)$ to obtain the difference equation analog to equation (2.12). We have

$$[C(t+2) - 2C(t+1) + C(t)][K_m + C(t)]^2 + [C(t+1) - C(t)]\{[K_m + C(t)]^2 (k_{21}-k_{12}) - V_mK_m\} + C(t)[K_m + C(t)]k_{12}V_m = 0 . \tag{2.13}$$

The model for estimating the rate constants is

$$[C(t+2) - 2C(t+1) + C(t)][K_m + C(t)]^2 + [C(t+1) - C(t)]\{[K_m + C(t)]^2 (k_{21}-k_{12}) - V_mK_m\} + C(t)[K_m + C(t)]k_{12}V_m = u(t) \tag{2.14}$$

where $u(t)$ is the error term with zero mean and appropriate variance.

Notice that the equation (2.14) is a polynomial with degree three in parameters $K_m$, $V_m$, $k_{12}$, and $k_{21}$ and results in a complicated

estimation procedure. With certain additional assumptions, such as those of some parameters known, nonlinear least-squares method may be utilized. The uniqueness of the resulting estimates is still questionable.

## 3. ESTIMATION OF RATE CONSTANTS

Several methods of estimation of rate constants in pharmacokinetics models are in common use. Numerical procedures leading to techniques using quasilinearization are due to Bellman and Kalaba (1965). Also computer programs such as NONLIN, by Metzler (1969) which is based on the modification of Gauss-Newton procedure are commonly used. We use the difference-equation approach discussed earlier for obtaining the estimates.

Using Runge-Kutta method, which is a part of NONLIN program, data are generated from the Michaelis-Menten model (2.1) with a given set of values of constants $K_m$ and $V_m$. A sample of 25 observations is generated and each value is increased by a standard normal random variate. The estimates of $V_m$ and $K_m$ are then made using the difference equations model of the Michaelis-Menten equations as in equation (2.4). The resulting estimates are given in Table I for one hundred such samples.

The frequency distributions of $\hat{K}_m$ and $\hat{V}_m$ are given in Tables II and III. Means and standard deviations for $\hat{K}_m$ and $\hat{V}_m$ are

$$\bar{K}_m = 0.056 \qquad \bar{V}_m = 0.242$$

$$s_k = 0.0452 \qquad s_v = 0.0495$$

To test the hypotheses that the sample of one hundred $\hat{K}_m$ and $\hat{V}_m$ arise from normal distributions, classical chi-squared test of goodness-of-fit and Lilliefors test are used, Conover (1971). The chi-squared values for the samples $\hat{K}_m$ and $\hat{V}_m$ are

$$\chi^2_{K_m} = 6.454 \text{ with 5 degrees of freedom,}$$

$$\chi^2_{V_m} = 2.516 \text{ with 4 degrees of freedom,}$$

which are not significant at 0.05 level.

For Lilliefors non-parametric test, the test statistic used is

$$T = \sup_x |F(x) - S(x)| ,$$

where F(x) is the standard normal distribution function and S(x) is the empirical distribution function. The values have been standardized using the means and standard deviations obtained above. Absolute values of (F(x)-S(x)) for both samples are recorded in Table I. The maximum value for $\hat{K}_m$ occurs at x = 0.23, where S(x) equals 0.53, F(x) equals 0.59, and T is 0.06. The maximum value for $\hat{V}_m$ occurs at x = -0.56, where S(x) equals 0.35, F(x) is 0.29 so that T equals 0.06. The maximum value 0.06 also occurs at other points, but at no point does the absolute difference of S(x) and F(x) exceed 0.06.

The Lilliefors test calls for rejection of our hypotheses at $\alpha = 0.20$ if T exceeds its 0.80 quantile. The critical region is obtained from tables in Conover (1971). The hypotheses are accepted, and therefore we conclude that the estimates $\hat{K}_m$ and $\hat{V}_m$ are normally distributed.

## ACKNOWLEDGEMENTS

The authors are grateful to Thomas Obremski for several discussions during the preparation of the paper.

TABLE I

| $K_m$ | $(K_m-.056)/.045$ | S(x)-F(x) | $V_m$ | $(V_m-.242)/.0495$ | S(x)-F(x) |
|---|---|---|---|---|---|
| 0.0 | -1.24 | .02 | 0.0415 | -4.05 | .01 |
| 0.0 | -1.24 | .02 | 0.1407 | -2.05 | .002 |
| 0.0 | -1.24 | .02 | 0.1508 | -1.84 | .002 |
| 0.0 | -1.24 | .02 | 0.1567 | -1.72 | .002 |
| 0.0 | -1.24 | .02 | 0.1611 | -1.63 | .001 |
| 0.0 | -1.24 | .02 | 0.1665 | -1.53 | .003 |
| 0.0 | -1.24 | .02 | 0.1797 | -1.26 | .03 |
| 0.0 | -1.24 | .02 | 0.1829 | -1.19 | .04 |
| 0.0005 | -1.23 | .01 | 0.1864 | -1.12 | .04 |
| 0.0027 | -1.18 | .01 | 0.1875 | -1.10 | .04 |
| 0.0045 | -1.14 | .01 | 0.1882 | -1.09 | .03 |
| 0.0055 | -1.12 | .01 | 0.1883 | -1.08 | .02 |
| 0.0058 | -1.11 | .003 | 0.1892 | -1.07 | .01 |
| 0.0078 | -1.07 | .002 | 0.1895 | -1.06 | .004 |
| 0.0100 | -1.02 | .01 | 0.1915 | -1.02 | .003 |
| 0.010 | -1.02 | .01 | 0.1938 | -0.97 | .006 |
| 0.0114 | -0.99 | .01 | 0.1945 | -0.96 | .002 |
| 0.0123 | -0.97 | .02 | 0.2020 | -0.81 | .03 |
| 0.0204 | -0.79 | .02 | 0.2037 | -0.77 | .02 |
| 0.0206 | -0.78 | .01 | 0.2039 | -0.77 | .02 |
| 0.0221 | -0.75 | .006 | 0.2044 | -0.76 | .01 |
| 0.0221 | -0.75 | .006 | 0.2056 | -0.74 | .009 |
| 0.0225 | -0.74 | .01 | 0.2061 | -0.73 | .003 |
| 0.0243 | -0.70 | .002 | 0.2072 | -0.70 | .002 |
| 0.0262 | -0.66 | .004 | 0.2095 | -0.66 | .005 |
| 0.0295 | -0.59 | .01 | 0.2101 | -0.64 | .01 |
| 0.0299 | -0.58 | .01 | 0.2102 | -0.64 | .01 |
| 0.0305 | -0.56 | .01 | 0.2107 | -0.63 | .03 |
| 0.0309 | -0.56 | .01 | 0.2108 | -0.63 | .03 |
| 0.0318 | -0.54 | .01 | 0.2130 | -0.59 | .04 |
| 0.0325 | -0.52 | .01 | 0.2130 | -0.59 | .04 |
| 0.0332 | -0.50 | .02 | 0.2130 | -0.59 | .04 |
| 0.0344 | -0.48 | .02 | 0.2131 | -0.58 | .06 |
| 0.0357 | -0.45 | .02 | 0.2135 | -0.58 | .06 |
| 0.0362 | -0.44 | .02 | 0.2144 | -0.56 | .06 |
| 0.0377 | -0.40 | .03 | 0.2162 | -0.52 | .05 |
| 0.0379 | -0.40 | .03 | 0.2205 | -0.43 | .04 |
| 0.0394 | -0.37 | .03 | 0.2210 | -0.42 | .04 |
| 0.0426 | -0.30 | .01 | 0.2217 | -0.41 | .05 |
| 0.0429 | -0.29 | .03 | 0.2232 | -0.38 | .05 |
| 0.0429 | -0.29 | .03 | 0.2264 | -0.32 | .04 |
| 0.0439 | -0.27 | .03 | 0.2329 | -0.18 | .01 |
| 0.0449 | -0.25 | .03 | 0.2330 | -0.18 | .01 |
| 0.0497 | -0.14 | .004 | 0.2330 | -0.18 | .01 |
| 0.0499 | -0.13 | .03 | 0.2350 | -0.14 | .006 |
| 0.0501 | -0.13 | .03 | 0.2354 | -0.13 | .01 |
| 0.0503 | -0.13 | .03 | 0.2360 | -0.12 | .040 |
| 0.0531 | -0.06 | .01 | 0.2360 | -0.12 | .04 |

TABLE I (continued)

| $K_m$ | $(K_m-.056)/.045$ | $S(x)-F(x)$ | $V_m$ | $(V_m-.242)/.0495$ | $S(x)-F(x)$ |
|---|---|---|---|---|---|
| 0.0558 | -0.00 | .01 | 0.2360 | -0.12 | .04 |
| 0.0567 | 0.02 | .008 | 0.2375 | -0.09 | .04 |
| 0.0583 | 0.05 | .009 | 0.2378 | -0.08 | .04 |
| 0.0610 | 0.11 | .02 | 0.2415 | -0.01 | .02 |
| 0.0662 | 0.23 | .06 | 0.2435 | 0.03 | .02 |
| 0.0667 | 0.24 | .02 | 0.2440 | 0.04 | .03 |
| 0.0668 | 0.24 | .02 | 0.2445 | 0.05 | .03 |
| 0.0668 | 0.24 | .02 | 0.2450 | 0.06 | .04 |
| 0.0670 | 0.24 | .02 | 0.2487 | 0.14 | .02 |
| 0.0674 | 0.25 | .02 | 0.2539 | 0.24 | .01 |
| 0.0678 | 0.26 | .01 | 0.2573 | 0.31 | .03 |
| 0.0689 | 0.29 | .01 | 0.2585 | 0.33 | .03 |
| 0.0699 | 0.31 | .01 | 0.2595 | 0.35 | .03 |
| 0.0740 | 0.40 | .03 | 0.2601 | 0.37 | .02 |
| 0.0758 | 0.44 | .04 | 0.2625 | 0.41 | .03 |
| 0.0764 | 0.45 | .03 | 0.2673 | 0.51 | .05 |
| 0.0769 | 0.46 | .02 | 0.2675 | 0.52 | .05 |
| 0.0805 | 0.54 | .03 | 0.2707 | 0.58 | .06 |
| 0.0806 | 0.54 | .03 | 0.2710 | 0.59 | .05 |
| 0.0824 | 0.58 | .03 | 0.2741 | 0.65 | .06 |
| 0.0827 | 0.59 | .03 | 0.2747 | 0.66 | .05 |
| 0.0841 | 0.62 | .03 | 0.2759 | 0.68 | .050 |
| 0.0844 | 0.63 | .02 | 0.2761 | 0.69 | .05 |
| 0.0853 | 0.65 | .02 | 0.2774 | 0.72 | .04 |
| 0.0880 | 0.71 | .03 | 0.2784 | 0.74 | .03 |
| 0.0885 | 0.72 | .02 | 0.2788 | 0.74 | .03 |
| 0.0895 | 0.74 | .02 | 0.2809 | 0.79 | .02 |
| 0.0906 | 0.77 | .01 | 0.2810 | 0.79 | .02 |
| 0.0906 | 0.77 | .01 | 0.2812 | 0.79 | .02 |
| 0.0909 | 0.77 | .01 | 0.2818 | 0.80 | .008 |
| 0.0925 | 0.81 | .001 | 0.2824 | 0.82 | .004 |
| 0.0946 | 0.85 | .002 | 0.2852 | 0.87 | .008 |
| 0.0952 | 0.87 | .01 | 0.2873 | 0.92 | .01 |
| 0.0984 | 0.94 | .006 | 0.2884 | 0.94 | .006 |
| 0.0994 | 0.96 | .001 | 0.2892 | 0.95 | .01 |
| 0.0998 | 0.97 | .01 | 0.2892 | 0.95 | .01 |
| 0.1001 | 0.98 | .02 | 0.2914 | 1.00 | .009 |
| 0.1028 | 1.04 | .01 | 0.2923 | 1.02 | .01 |
| 0.1043 | 1.07 | .02 | 0.2974 | 1.12 | .003 |
| 0.1090 | 1.17 | .01 | 0.3006 | 1.18 | .001 |
| 0.1137 | 1.28 | .01 | 0.3032 | 1.24 | .003 |
| 0.1138 | 1.28 | .01 | 0.3052 | 1.28 | .001 |
| 0.1143 | 1.29 | .01 | 0.3057 | 1.29 | .02 |
| 0.1169 | 1.35 | .01 | 0.3058 | 1.29 | .01 |
| 0.1178 | 1.37 | .02 | 0.3094 | 1.36 | .02 |

TABLE I (continued)

| $K_m$ | $(K_m - .056)/.045$ | S(x)-F(x) | $V_m$ | $(V_m - .242)/.0495$ | S(x)-F(x) |
|---|---|---|---|---|---|
| 0.1201 | 1.42 | .02 | 0.3114 | 1.40 | .02 |
| 0.1206 | 1.43 | .03 | 0.3161 | 1.50 | .02 |
| 0.1222 | 1.46 | .04 | 0.3233 | 1.64 | .01 |
| 0.1232 | 1.49 | .04 | 0.3248 | 1.67 | .02 |
| 0.1303 | 1.64 | .04 | 0.3250 | 1.68 | .03 |
| 0.1311 | 1.66 | .04 | 0.3283 | 1.74 | .04 |
| 0.1322 | 1.69 | .05 | 0.3333 | 1.84 | .04 |

TABLE II

FREQUENCY DISTRIBUTION OF $\hat{K}_m$

| $\hat{K}_m$ | Frequencies | Relative Frequencies | Cumulative Relative Frequencies |
|---|---|---|---|
| 0 | 8 | 0.08 | 0.08 |
| 0.00-0.02 | 10 | 0.10 | 0.18 |
| 0.02-0.04 | 20 | 0.20 | 0.38 |
| 0.04-0.06 | 13 | 0.13 | 0.51 |
| 0.06-0.08 | 14 | 0.14 | 0.65 |
| 0.08-0.10 | 19 | 0.19 | 0.84 |
| 0.10-0.12 | 9 | 0.09 | 0.93 |
| 0.12-0.14 | 7 | 0.07 | 1.0 |
| Total | 100 | 1.0 | |

TABLE III

FREQUENCY DISTRIBUTION OF $\hat{V}_m$

| $\hat{K}_m$ | Frequencies | Relative Frequencies | Cumulative Relative Frequencies |
|---|---|---|---|
| 0.00–0.17 | 6 | 0.06 | 0.06 |
| 0.17–0.20 | 11 | 0.11 | 0.17 |
| 0.20–0.23 | 24 | 0.24 | 0.41 |
| 0.23–0.26 | 20 | 0.20 | 0.61 |
| 0.26–0.29 | 23 | 0.23 | 0.84 |
| 0.29–0.32 | 11 | 0.11 | 0.95 |
| 0.32–0.35 | 5 | 0.05 | 1.0 |
| Total | 100 | 1.0 | |

## 4. REFERENCES


1. Bellman, Richard and Kalaba, Robert, Quasilinearization and Nonlinear Boundary-value Problem, American Elsevier Company, New York (1965).

2. Conover, W. J., Practical Nonparametric Statistics, John Wiley, New York, 1971.

3. Metzler, C. M., A User's Manual for NONLIN, Technical Report, Upjohn Company, Kalamazoo, Michigan (1969).

4. Michaelis, L. and Menten, M. L., Die Kinetik der Invertinwirkung, Biochem. Z., 49, 333-369 (1913).

5. Rustagi, J. S. and Singh, U., Statistical Analysis of Compartmental Models with Applications to Pharmacokinetics and Bioavailability, Application of Statistics (P. R. Krishniah, Editor), (1977) 461-480.

6. Sedman, A. J. and Wagner, J. G., Importance of the Use of the Appropriate Pharmacokinetics Model to Analyse in vivo Enzyme Constants, J. Pharma. and Biophar., 2 (1974), 161-173.

7. Wagner, J. G., Biopharmaceutics and Relevant Pharmacokinetics, Hamilton Press, Illinois (1971).

8. Wagner, J. G., Properties of Michaelis-Menten equation and its integrated form which are useful in pharmacokinetics, J. Pharma. and Biophar., 1 (1973), 103-121.